REMARKS ON SOME EXPERIMENTS WITH SNAKE-VENOM.*

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The experiments were undertaken: (1) In order to ascertain whether certain South African snakes, about which there were doubts till now, are harmless or poisonous (specially Opistoglypha): (2) To study the pathological-anatomical lesions provoked by the venom of South African snakes.

1. Aglypha.†

An emulsion of both maxillary glands in normal saline solution of *Boodon lineatus*, the brown house-snake, was injected subcutaneously into a rabbit and a guinea-pig.

Result.—No local, nor general pathological alterations appeared.

With another portion of the emulsion experiments for hæmolysis were tried with original human blood and horse blood, previously washed with normal saline solution.

No hæmolysis took place within 8 hours' stay in the incubator.

2. Opistoglypha.

(*a*) A specimen of *Leptodira hotambœia*, red-lip snake, was forced to bite the ear of a guinea-pig.

As no symptoms made their appearance, the snake was killed and an emulsion of both maxillary glands injected into the same guinea-pig subcutaneously. After $5\frac{1}{2}$ hours the guinea-pig was found dead with its mouth full of forage.

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† From a toxicological standpoint the classification of the snakes in: 1. Aglypha, 2. Opistoglypha, 3. Proteroglypha, 4. Solenoglypha, is the most satisfactory one, as will be shown in the course of the paper.

Post Mortem.—Blood partially coagulated. Heart in diastole. Endocard normal, lungs normal, stomach filled, normal; all other abdominal organs without any lesions.

Conclusion.—The venom of *Leptodira hotambœia*, or, better, its effective constituent, must have been absorbed by the nervous system.

In another instance an emulsion of the maxillary glands of the red-lip snake did not provoke any sign of illness, when injected under the skin of a rabbit. The venom appears therefore to be more virulent for guinea-pigs than for rabbits, if the snake itself were under normal conditions.

(*b*) *Tarbophis semiannulatus*. An emulsion of both maxillary glands of a young specimen produced no pathological appearances after subcutaneous injection into a small guinea-pig.

(*c*) An emulsion of the right maxillary gland of *Trimerorhinus tritæniatus* (Günther), striped "schaap-steekker," subcutaneously injected, killed a small guinea-pig (weight 150 gr.) in 15 minutes.

Symptoms.—Salivation, masticatory movements, secretion from the lachrymal glands.

Post Mortem.—Nothing particular.

(*d*) *Aparallactus capensis*. Black-headed snake. An emulsion of the maxillary glands, subcutaneously injected into a small guinea-pig, produced no symptoms of illness.

(*e*) *Psamophis sibilans*. Hissing sand-snake. An emulsion of maxillary glands of 3 individuals injected subcutaneously into (1) a frog (*Rana delalandi*); (2) a small rabbit; and (3) a small guinea-pig (weight 310 gr.). The frog and the rabbit did not become sick at all. The guinea-pig showed no symptoms for 50 minutes. After this time its respiration became quick and spasmodic. The animal laid down; cramps of the hind legs and retarded respiration were the final symptoms. Death occurred 1 hour after injection.

Post Mortem.—No lesions.

3. Proteroglypha.

(*a*) Five drops of venom from *Dendraspis angusticeps*, Mamba, were diluted with 1·5 c.c. 0·9 per cent. solution of NaCl, and the mixture was divided into 3 parts and injected into 3 rabbits (2 subcutaneously, 1 partially subcutaneously, partially intravenously).

Symptoms.—First rabbit. Quick jerky respiration, extension of the head, salivation, discharge of big white tears, cramps of the legs, paralysis; death after 30 minutes.

Second rabbit. Extension of the head, abundant salivation, jerky respiration, ataxy of hind quarters, stupid running forwards, cramps of the legs, paralysis; death after 15 minutes.

Third rabbit. Accelerated respiration, discharge of white tears, salivation, slight prolapsus vaginæ, output of white urine, cramps, paralysis; death after 25 minutes. Microscopical examination of the saliva: No leucocytes but small drops of a yellow liquid, like an oil.

Post Mortem.—In all three rabbits: Hæmorrhagic infiltration of the place of injection, blood thin somewhat hæmolytic; no abnormalities in the internal organs.

(*b*) *Naia haie.* Cobra. One drop of poison, dried out for 2 days, was redissolved in 2 c.c. normal saline solution and injected under the abdominal skin of a rabbit.

Symptoms.—Uneasiness; accelerated, sometimes jerky respiration for the first 15 minutes; emission of urine. The animal then became quiet and died 35 minutes after injection without any additional symptoms.

Post Mortem.—Blood dark; heart in diastole; ecchymoses on left endocardium, right endocardium normal; lungs collapsed, œdematous; hæmoglobin-stained liquid in peritoneal cavity; omentum, mesentery, and serous coat of small intestines injected. One Peyer's patch of cœcum swollen and injected, other abdominal organs normal. Urinary bladder slightly injected, urine normal. Very likely the dose was not large enough to produce more distinct symptoms.

4. Solenoglypha.

(*a*) A specimen of *Causus defilippi*—night-adder—was allowed to bite into the hind leg of a guinea-pig. The latter showed uneasiness and spasmodic respiration, but was normal again after an hour's time.

A similar second experiment with both the same, snake and guinea-pig, was without result. The night-adder is known as a poisonous snake.

(*b*) *Bitis orietans.* Puff-adder. The poison was squeezed out and $1\frac{1}{2}$ drops of it injected—

1. Under the abdominal skin of a guinea-pig.

Symptoms.—Uneasiness, whimpers from time to time; the animal was paralytic 2 hours after injection, and was found dead $5\frac{1}{2}$ hours later.

Post Mortem.—Necrosis of the skin on the spot of the injection, diffuse dark red hæmorrhagic infiltration of the skin and subcutaneous tissue and musculature of the abdomen. Blood thin, hæmolytic. Uterus pregnant; uterine liquid, stained with hæmoglobin.

2. A rabbit was injected, like the guinea-pig, with 1·5 drops of the poison of the same puff-adder. Only local symptoms were seen, consisting in a necrosis of the skin where the injection had been made, about 3 cm. in diameter. The wound was not completely healed after 12 days.

This venom was hæmolytic *in vitro* for horse blood as is proved by the following experiments :—

Tube Number	I.	II.	III.	Control.
0·9 per cent. NaCl	1 c.c.	1 c.c.	1 c.c.	1 c.c.
Snake-poison diluted with 0·9 per cent. NaCl 1 : 4	15 drops	20 drops	20 drops	0
Blood, 5 drops	washed horse blood	unwashed horse blood	unwashed ox blood	unwashed ox blood
Result after 45 minutes at 37° Cels.	0	0	0	0
Result after 6 hours at 25° Cels.	0	complete hæmolysis	distinct hæmolysis	0
Addition of horse serum	1 c.c.	—	—	—
Result after 3 hours at 37° Cels.	almost complete hæmolysis	—	—	—

Snake-venom diluted with NaCl 1 : 4.

In each tube 1 c.c. NaCl solution and 0·5 c.c. 5 per cent. emulsion of ox blood 3 times washed.

Tube Number	I.	II.	III.	IV.	V.	Control.
Poison solution	3 drops	5 drops	8 drops	10 drops	12 drops	0
Result after 8 hours' delay at room temp.	0	0	0	0	0	0
Follows	centrifug.	centrifug.	—	—	centrifug.	—
Added to the liquid unwashed ox blood	0·5 c.c.	0·5 c.c.	—	—	0·5 c.c.	—
Result after 3 hours at 37° Cels.	0	0	—	—	0	—
Added ox serum	to the deposit 1 c.c.	to the deposit 1 c.c.	1 c.c.	1 c.c.	to the deposit 1 c.c.	—
Result after 3 hours at 37° Cels.	0	0	0	0	0	—

Snake-venom diluted with NaCl 1 : 4.

Each tube contains 1 c.c. NaCl + 0·5 c.c. per cent. emulsion of horse blood 3 times washed.

Tube Number	I.	II.	III.	IV.	V.	Control.
Venom solution	3 drops	5 drops	8 drops	10 drops	12 drops	0
Result after 8 hours' delay at 25° Cels.	0	0	0	0	0	0
Follows	—	—	centrifug.	centrifug.	—	—
Added unwashed horse blood to the liquid	—	—	0·5 c.c.	0·5 c.c.	—	—
Result after 3 hours at 37° Cels.	—	—	almost complete hæmolysis	almost complete hæmolysis	—	—
Added horse serum	1 c.c.	1 c.c.	1 c.c. to the deposit	1 c.c. to the deposit	1 c.c.	—
Result after 3 hours at 37° Cels.	slight hæmolysis	slight hæmolysis	distinct hæmolysis	complete hæmolysis	distinct hæmolysis	—

These experiments demonstrate :—

(*a*) That original blood is easier dissolved by the puff-adder poison than previously washed blood; that is to say, blood corpuscles without serum.

(*b*) That horse blood is more susceptible than ox blood.

(*c*) That the venom is absorbed by the horse-blood corpuscles and emphasises itself after addition of horse serum.

Hence the venom alone has no hæmolytic power. It contains a com-

ponent, which, associated with a serum component (horse), dissolves horse-blood corpuscles. According to numerous experiments with other snake-poisons the serum component in question is lecithine, and there is very little doubt that also in our experiments lecithine acts as an activator for the hæmotoxic component of the puff-adder poison, forming with it the hæmolytic lecithide.

3. 0·3 c.c. puff-adder poison were injected subcutaneously into a horse 3081, and the following *symptoms* recorded :—

Uneasiness; discharge from nostrils; pulse 15 minutes after injection, 60; respiration, 70; 35 minutes after injection, pulse 60; respiration, 38. Enormous swelling of the place of injection. Trembling of pectoral, gluteal and caudal muscles. The temperature rose 6 hours after injection up to 105·0, but fell within an hour to 102·4. The horse died 9 hours after injection.

Post Mortem.—Enormous swelling on left side, where inoculation took place, extending from base of neck and pectoral muscles to the last rib, corresponding with œdematous and hæmorrhagic infiltration of subcutaneous and intermuscular tissues in these regions. Liquid in pleural cavity and pericardium slightly increased. Hæmorrhagic spots on anterior side of diaphragm, on pericardium and left costal pleura, ecchymoses on visceral pleura, hæmorrhagic infiltration of mediastinal glands, ecchymoses on epicardium and both endocardia. Liver khaki-coloured, with numerous hæmorrhages in the capsula. Spleen and kidneys pale. Mucosa of urinary bladder showed patchy hyperæmia and petechieæ; urine normal. Bone marrow of left radius and femur normal. Hæmorrhagic infiltration of perispinal tissue near seat of inoculation.

Résumé.

With regard to the question which of the doubtful South African snakes, chiefly Opistoglypha, are poisonous or harmless, only a preliminary answer can be given, as the experiments are not yet numerous enough. Especially the experiments which gave negative results have to be repeated.

Boodon lineatus (Aglypha) proved in two instances to be harmless, as was to be expected. The experiments with emulsions of maxillary glands from Opistoglypha gave the following results :—

Leptodira hotambœia was poisonous in one, harmless in another case.

Tarbophis semiannulatus was harmless (in one instance).

Trimerorhinus tritæniatus proved to be poisonous (in one case).

Aparallactus capensis was harmless (in one case).

Psamophis sibilans was poisonous in one, harmless in two instances, one of the latter being an amphibian.

That is to say, three of five snakes belonging to the family Opistoglypha are apparently not harmless.

A reasonable objection might be made against the method of inoculating the maxillary gland substance itself instead of their secretion, namely, that emulsions of any mammalian organ have toxic effect on mammalian organisms, and that reptilian albuminoids must be still more toxic. Indeed, control experiments with other organs of the snakes in question have to be made; the best proof as to whether a snake is harmless or poisonous is certainly the injection of the secretion of the maxillary glands.

But, on the other hand, it is not very likely that an emulsion of maxillary glands would provoke symptoms so similar to those that signify a real snake-poison, and it is not easy to understand why every emulsion had not a toxic effect (emulsion from *Boodon* and *Tarbophis* were completely without effect).

Finally, I should like to mention as a corroboration of this opinion that the Opistoglyphal snakes, the emulsions of which were poisonous, have longer grooved teeth than those yielding a harmless gland emulsion.

The *symptoms* in all animals injected with the poisons of Opistoglypha, Proteroglypha, and Solenoglypha were about the same, namely as follows :—

Uneasiness; accelerated, sometimes spasmodic respiration; salivation; cramps; finally paralysis and retarded respiration antecedent to death.

That is to say, the first symptoms are expressions of nervous excitement, finally followed by paralysis. In this respect the symptoms resemble those of rabies and narcotics.

It would be very useful in practice to be able to point out the family to which a snake belongs from the clinical symptoms produced by its poison. As it has to be deduced from numerous experiments in other countries, the antisera against South Africa snake-venom would be specific for each of the various families of snakes. In a concrete case of snake-bite the knowledge of the zoological classification of the snake would determine which serum has to be applied for the treatment.

As only the adequate or specific serum is efficacious, the selection of this serum (among a number of snake antisera) for the application must be of great importance.

According to our experiments, there is a difference between the symptoms of Opistoglypha venom and Solenoglypha venom, namely, the poison of Opistoglypha produced no local alterations or very slight ones, while the Solenoglypha poison caused hæmorrhagic infiltrations, swellings, and necrosis of the skin.

The difference in the *post-mortem lesions* produced by the three venomous families of snakes are the following :—

1. *Opistoglypha.*—No pathological anatomical alteration at all; the poison is a simple one, a mere *neurotoxine.*

2. *Proteroglypha.*—Local hæmorrhages, slight hæmolysis; the poison consists of two components—a *neurotoxine* and a *hæmotoxine.*

3. *Solenoglypha.*—Local hæmorrhages and necrosis, hæmolysis. Besides the *neurotoxine* there is a *strong hæmotoxine*, which dissolves red blood corpuscles. It is yet doubtful whether the necrosis is due to a special component of the poison.

Perhaps the hæmolysine is able to destroy besides erythrocytes other cells in the same manner as hæmolytic sera, which at the same time cause necrosis.

With regard to the question as to whether snakes themselves are susceptible to snake-poisons, we made one experiment.

0·25 c.c. original poison of *Bitis arietans* were injected partially subcutaneously, partially intraperitoneally into a large ♀ specimen of *Ablabophis rufulus*—water-snake—which is non-poisonous.

Symptoms.—Besides a slight excitement nothing particular the first day. The next day, however, the places of injection were swollen with hæmorrhagic margins, and the epidermis came off; scales mollified. The snake died the following night, *i.e.*, about 36 hours after injection.

Post Mortem.—The symptoms were the same as in mammalians, namely, infiltration of cutis and costal muscles round the places of injection, hæmorrhagic spots on fat bodies and on the intestines. Liver and kidneys injected. Blood in heart not coagulated-hæmolytic.

The water-snake possesses therefore not absolute but considerable immunity against the poison of the puff-adder, compared with the horse that received practically the same doses—the weight of the horse is about 500 times greater, and it died within 9 hours.

I here take the opportunity of thanking Dr. L. H. Gough for his most valuable assistance in carrying out these experiments.